



Analysis of expression of mRNA Matrix Metalloproteinase (MMP)-8 gene and Tissue Inhibitors of Metalloproteinase (TIMP)-1 in Intraperitoneal Adhesion after Usage of Hyaluronate acid-Carboxymethylcellulose (HA-CMC) or Virgin Coconut Oil (VCO)

Jimmy Panelewen¹, Aryono Djuned Puspongoro², Ibrahim Labeda³, Andi Asadul Islam³, Mochammad Hatta^{4,*}

¹Department of Surgery, Faculty of Medicine, Sam Ratulagi University, Manado, Indonesia

²Department of Surgery, Faculty of Medicine, Indonesia University, Jakarta, Indonesia

³Department of Surgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

⁴Molecular Biology and Immunology Laboratory, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

*Corresponding author: hattaram@indosat.net.id

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Abstract Background: Purpose : To understand the expression of matrix metalloproteinase (MMP)-8 mRNA gene and tissue inhibitors of metalloproteinase (TIMP)-1 in normal peritoneum tissue and intraperitoneal adhesion tissue after the usage of virgin coconut oil (VCO) and hyaluronate Acid-carboxymethylcellulose (HA-CMC). **Methods:** The research is using an experimental lab rat "*rattus norvegicus strain wistar*" which is divided into 3 groups. Laparotomy (pre) is done to gain parietal peritoneum tissue at the lower left quadrant of the abdomen, in order to obtain the expression of mRNA gene MMP-8 and TIMP-1. First group was control group, second group was treated with VCO, while the third group was treated with HA-CMC. After two weeks period, another relaparotomy (post) was held to obtain the adhesion tissue and to run another examination on the MMP-8 mRNA gene expression and TIMP-1. **Results:** There is a meaningful decrease for the MMP-8 mRNA gene expression in the peritoneum tissue for the control group at the pre and post laparotomy ($p < 0,001$). There is a meaningful over expression of TIMP-1 mRNA gene expression at pre dan post laparotomy of control group ($p < 0,001$). While on the other hand, at the VCO nor HA-CMC group, there is an insignificant increase of MMP-8 mRNA gene expression ($p = 0,075$ and $p = 0,791$) and insignificant decrease of TIMP-1 mRNA gene expression ($p = 0,015$ and $p = 0,368$). **Conclusion:** The decrease of MMP-8 mRNA gene expression and the increasing of TIMP-1 mRNA gene expression of control group shows a high risk of adhesion incidence. Although it is not statistically meaningful, the result of MMP-8 mRNA and TIMP-1 mRNA gene expression of VCO and HA-CMC indicates that there is a decrease of intraperitoneal adhesion risks, and the nature of VCO anti adhesive compared to HA-CMC.

Keywords: intraperitoneal adhesion, MMP-8 mRNA gene, TIMP-1 mRNA gene, VCO, HA-CMC

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1. Introduction

Intraperitoneal adhesion could happen in every operation by opening the abdomen, with the incidence of 67-93%, even up to 97% for operation of the pelvic area [10]. The complications can be seen as a clinical symptoms of intestinal obstruction, infertility or chronic pain in pelvic region [4,20]. For more than 342.000

operation was done to release the adhesion in 2004 [32] with the budget up to 1,3 billion US dollars in United States and up to 13 billion US dollars in Sweden for every year [30].

Definition of intraperitoneal adhesion is a bond of tissues band which is well vascularized and innervated, where those tissue bands bond or connect intraperitoneal organs which are normally separated [2,29]. Pathogenesis of adhesion involves the inflammation process, coagulation, fibrinolysis, and degradation of extracellular

matrix. The healing process without adhesion could happen if fibrinolysis and degradation of fibrin matrix is in balance. Degradation of matrix which is done by MMP and inhibited by TIMP. After being activated by plasmin, MMP is going to be able to degrade all ECM component [3,7,8,12,13,31].

Many efforts has been done to decrease the risk of adhesion, starting from the *no touch* operation technique, until the usage of antiadhesive agents [8,31]. Hyaluronic acid (HA) is a popular agent combined with carboxymethylcellulose (CMC). HA plays a role in stabilizing extracellular matrix which in turn interact with the cell surface to effect the normal activity of the cell itself [24]. Due to its viscosity, HA is a lubricant like agent which in turn will cause the intestines floats (hydroflotation) and keep them separated one another [22,25] CMC also prevents adhesion by separating the serosal surface of intestines and minimizing trauma [1,9,23].

Virgin coconut oil (VCO) is a substance extracted from pure coconut meat without boiling process, and can act as anti-inflammatory, anti-thrombotic, and also anti-oxidant [17,27].

2. Materials and Method

Fifteen male *rattus norvegicus strain wistar* with the age of 2 months old, and weight of 125-150 grams are divided

into three groups. First group acts as control group, second group was given 1cc of VCO intraperitoneal, and the third group was given 1 cc of HA-CMC intraperitoneal. Two laparotomies was held for every mouse, at pre an post treatment. The anesthesia was using ketamine 6-10 mg/kg and midazolam 70-80 mcg/kg, both were given intramuscular. The first laparotomy was to take sample of parietal peritoneum tissue at the left lower quadrant of the abdomen, at the second group the procedure was followed by the usage of VCO, and the usage of HA-CMC for the third group. After two weeks period, a relaparotomy was held to take samples of adhesion tissues. The peritoneum and the adhesion tissues was examined for the MMP-8 mRNA gene expression and ekspresi mRNA gen TIMP-1 mRNA gene expression, using the *quantitative reverse polymerase chain reaction* (qRT-PCR).

Statistic analysis was held using SPSS 20 *software*. The final result was analyzed using t-test.

3. Results

The examination of mRNA sample gene expression for pre and post laparotomy was done up to three times (triplicate), and from that results the median and mean mark was acquired. The sample characteristic on 3 groups was descriptically analyzed, which can be seen on Table 1.

Table 1. Descriptive analysis result of research variables

Variabels	Group	Pre		Post	
		Min/Max (median)	Mean±SD	Min/Max (Median)	Mean±SD
MMP8 mRNA gene expression	Control (n=5)	7,04/7,21 (7,11)	7,12 ± 0,06	5,26/5,39 (5,29)	5,31 ± 0,05
	VCO (n=5)	7,05/7,25 (7,16)	7,14 ± 0,09	7,01/10,80 (10,700)	9,30 ± 2,00
	HA-CMC (n=5)	7,08/7,19 (7,18)	7,16 ± 0,05	5,30/8,07 (8,00)	7,31 ± 1,19
TIMP-1 mRNA gene expression	Control (n=5)	9,96/10,16 (10,030)	10,03 ± 0,08	11,88/12,06 (12,00)	11,98 ± 0,07
	VCO (n=5)	10,04/10,30 (10,20)	10,18 ± 0,09	7,01/10,24 (7,15)	7,74 ± 1,40
	HA-CMC (n=5)	10,01/10,19 (10,09)	10,10 ± 0,07	8,04/12,15 (8,07)	9,27 ± 1,82

Table 2 showed a meaningful decrease of MMP-8 mRNA gene expression as many as 1,81 ($p < 0,05$) from ($7,12 \pm 0,06$) down to ($5,31 \pm 0,05$); while on the VCO group there was an increase as many as 2,16 from ($7,14 \pm 0,09$) up to ($9,30 \pm 2,00$) and for the HA-CMC group there was a increase of 0,15 from ($7,16 \pm 0,05$) up to ($7,31 \pm 1,19$), but it was not a meaningful increase ($p > 0,05$). At

the control group there is an increased MMP-8 mRNA gene expression, but on the other hand, both treated groups seems to have a decreased MMP-8 mRNA gene expression. This can be observed from the graphic below that shows a decrease of MMP-8 mRNA gene expression in control group and increasement at both treated group especially VCO group.

Estimated Marginal Means of MEASURE_1

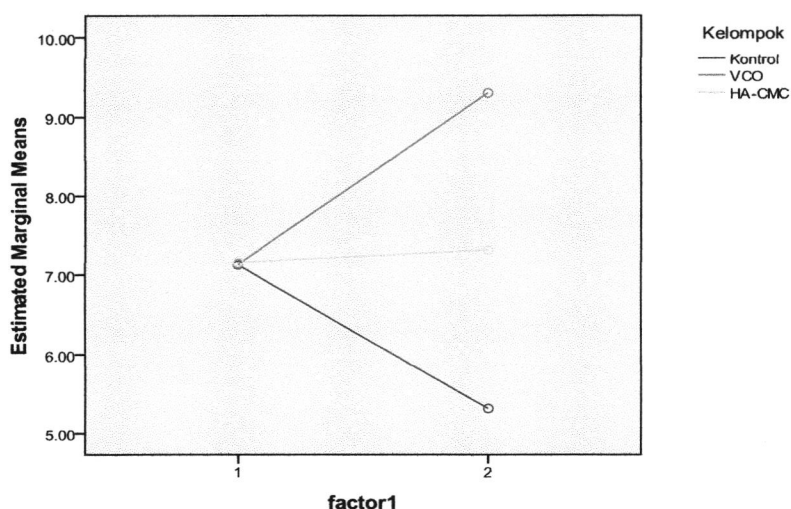


Table 2. MMP-8 mRNA gene expression differences on all three groups

Groups	Eksresi mRNA gen MMP-8 (log)			P Result
	Pre	Post	Differences	
Control (n=5)	7,12 ± 0,06	5,31 ± 0,05	-(1,81 ± 0,09) ^a	<0,001
VCO (n=5)	7,14 ± 0,09	9,30 ± 2,00	(2,16±2,03) ^b	=0,075
HA-CMC (n=5)	7,16 ± 0,05	7,31 ± 1,19	(0,15±1,20) ^b	=0,791

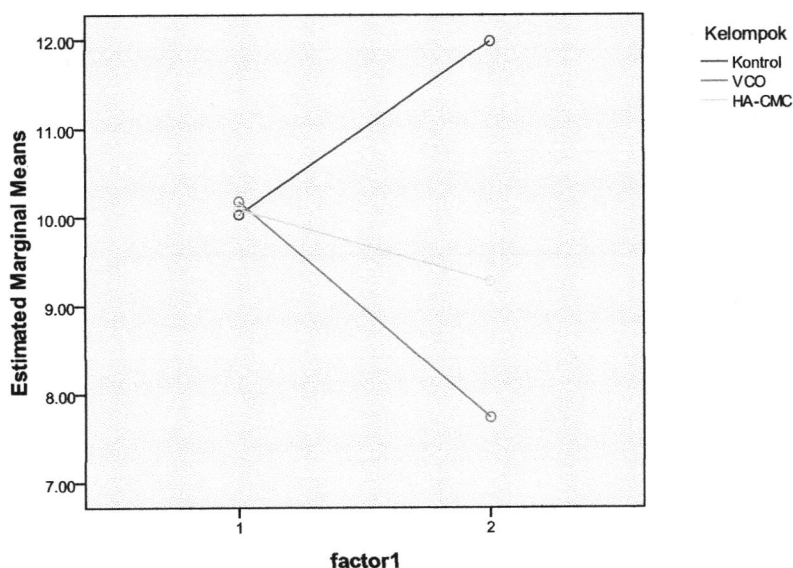
Table 3 showed that TIMP-1 mRNA gene expression at control group has a meaningful increase as many as 1,95 ($p < 0,05$) from $(10,03 \pm 0,08)$ up to $(11,98 \pm 0,07)$; whilst at VCO group there was a meaningful decrease as many as 2,44 ($p < 0,05$) from $(10,18 \pm 0,09)$ down to $(7,74 \pm 1,40)$. At

HA-CMC there was a decrease of 0,82 from $(10,10 \pm 0,07)$ down to $(9,27 \pm 1,82)$, but it was not a meaningful one ($p > 0,05$). At control group the TIMP-1 mRNA gene expression was decreasing, but was increasing at both treated groups, especially the one given VCO.

Table 3. TIMP-1 mRNA gene expression differences on all three groups

Group	TIMP-1 mRNA gene expression			P result
	Pre	Post	Differences	
Control(n=5)	10,03 ± 0,08	11,98 ± 0,07	(1,95±0,08) ^a	<0,001
VCO(n=5)	10,18 ± 0,09	7,74 ± 1,40	-(2,44±1,33) ^b	=0,015
HA-CMC(n=5)	10,10 ± 0,07	9,27 ± 1,82	-(0,82±1,81) ^b	=0,368

Estimated Marginal Means of MEASURE_1



4. Pembahasan

MMP is produced by peritoneum mesotel cells and regulated by interleukin-1 and TGFβ [2]. MMP is controlled at the protein level through the activator, inhibitor, or the location of cell surface. The MMP expression is usually low at adult normal tissue. MMPs which are regulated at the transcription and post transcription becomes upregulated if there is disturbances in the system such as wound healing or remodelling of sick tissue [6]. MMP-8 is specifically produced by the neutrophil specific granule [19], but also expressed by many different kind of cells such as epithelial cell, fibroblast, macrophage, and endothelial cells [28].

Many documented the relation between peritoneum and adhesion tissue to MMP and TIMP. The concentration of TIMP is higher in fibrotic adhesion tissue compared to normal serosal tissue of peritoneum. The increasing expression of TIMP-1 at fibrotic adhesion tissues is parallel to the expression of TGF-β1. At in vitro condition, it will decrease the MMP expression and increase the TIMP expression, which in turn will decrease the matrix

degradation and increase fibrotic tissue [29]. Induction of TIMP-1 as a response from *growth factor* and cytokine, is in opposite with the induction of MMP, but is in accordance with the induction of TIMP-1 and TIMP-3 activity. In general, the balance between TIMP and MMP is needed to regulate the extracellular matrix [14].

Research with *level of evidence base grade A*; the usage of HA-CMC can decrease adhesion rate, but not useful in managing *reformation adhesion* or adhesion recurrences. [11]. Study on male rat, comparing the effect of HA-CMC with control group given NaCl 0,9%, shows that adhesion level on HA-CMC group decreased by 40% and statistically meaningful with $p = 0,0382$, but no mean compared to group treated with *phosphatidylcholine* or group given tPA with $p > 0,05$ [15]. Although this product has been used widely, but questions still remain surrounding the efficiency and side effects [5,16,21].

Study of VCO usage in treating intraperitoneal adhesion has never been done before, but the effect of VCO as its mechanic effect as a lubricant and the effect of monolaurin anti-inflammatory as a monoester form of lauric acid. The activity of lauric acid monoglyceride (monolaurin) as antimicrobes has been reported since 1966, where the site of activity is at the DNA and RNA

envelope of viral or bacteria. Some viruses such as HIV, pox, Herpes Simplex-1, vesicular stomatitis, visna virus, and cytomegalovirus become inactive with the usage of monolaurin [18]. Minor components of VCO such as tocopherol or vitamin E, are anti oxidants which act as anti inflammation [26].

5. Conclusion

This study showed that VCO and HA-CMC can regulate the balance of MMP-8 and TIMP-1 mRNA gene expression, which provide the probability of curing the peritoneum without adhesion. As seen from the result above, the VCO role is more dominant compared with HA-CMC in MMP-8 and TIMP-1 mRNA gene expression.

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